## REMARKS

Favorable reconsideration of the subject application, as amended above, is respectfully requested in view of the comments below.

Claims 9-29 are pending in the present application. Claims 10-12 and 19-29 were previously withdrawn from consideration; and claims 1-8 were previously canceled. Accordingly, claims 9 and 14-18 are presented for examination on the merits.

### I. Objection to Claim 18

It is respectfully submitted that the objection to claim 18 is rendered moot by the amendment to the claim.

### II. Rejection of Claims 14-17 Under 35 U.S.C. § 112, Second Paragraph

It is respectfully submitted that the amendments to the claims render this ground of rejection moot.

### III. Rejection of Claims 9 and 13-17 Under 35 U.S.C § 112, First Paragraph

Claims 9 and 13-18 stand rejected under 35 U.S.C § 112, first paragraph. The Examiner states that the specification is enabling for enzymes having SEQ ID NO. 1, 3 and/or 5 and having a single specified substitution at position 251 and capable of hydrolyzing organophosphates. The Examiner asserts, however, that the specification does not enable any such enzymes encoded by polynucleotides having at least 80% homology to the polynucleotides encoding the above-referenced enzymes.

Applicants respectfully disagree with the Examiner's assertions.

It appears from the Examiner's remarks on pages 6-7 of the Ofice Action that the Examiner has confused the claimed polypeptide sequence (SEQ ID NO. 8), which is a recombinant *L. cuprina* enzyme, with the *M. domestica* sequence. To clarify, the claims are directed to the recombinant *L. cuprina* sequence (SEQ ID NO. 8) and sequences sharing 75% similarity thereto, which encodes organophosphate resistance.

The present claims are directed to recombinant enzymes having the specified activity (function), having a Leu, Ala, Ser, ILe, Val, Thr, Cys, Met or Gly residue at position 251 and which have at least about 75% sequence identity with SEQ ID NO. 8 (specified structure). The specification shows through sequence alignments that the claimed recombinant protein conferring malathion resistance can tolerate up to at least 25% sequence variation. The sequence alignments also demonstrate that it is essential for malathion resistance that the tryptophan at position 251 is replaced with a less bulky amino acid, i.e., Leu, Ser, Ala, Ile, Val, Thr, Met or Gly. Thus, the specification clearly demonstrates to one of skill in the art that numerous amino acid substitutions may be made while retaining the claimed enzyme activity. One of skill in the art at the time of the invention could readily introduce amino acid changes into the sequence based on the data provided in the specification, and expect to obtain functional enzymes having the claimed specificity, without undue experimentation. In particular, one of skill in the art at the time was capable of utilizing the disclosed alignment data to make conservative amino acid substitutions in the claimed recombinant enzyme.

Furthermore, the claimed recombinant enzyme is readily obtainable by performing nucleic acid hybridization with a probe having a sequence of any of the disclosed allele sequences under appropriate hybridization conditions, and expressing the protein and testing for activity as taught in the specification. One of skill in the art is capable of obtaining polynucleotide sequences encoding the claimed polypeptide. Indeed, claims to such polynucleotides encoding the enzyme have issued in US 6,235,515 (the parent application).

The specification teaches how to obtain the DNA encoding the claimed polypeptides, and teaches how to test for the claimed activity. The specification also provides several examples of recombinant enzymes as claimed, obtained from different species. Using the methods set forth in the specification, Applicants obtained several recombinant polypeptides having the claimed activity from different species and exhibiting up to 25 % amino acid variation.

Accordingly, the rejection of claims 9 and 13-18 under 35 U.S.C § 112, first paragraph is respectfully traversed.

## IV. Rejection of Claims 9 and 13-18 Under 35 U.S.C § 102(b)

Claims 9 and 13-18 stand rejected under 35 U.S.C § 102(b) over Whyard et al., Pesticide Biochemistry and Physiology (Whyard a) or Whyard et al., Biochemical Genetics (Whyard b). The Examiner states that the cited prior art discloses a naturally occurring (non-recombinant protein) having the claimed sequence and activity.

This rejection is respectfully traversed as follows.

The enzyme that Whyard et al. isolated from *L. cuprina* is not the same as the organophosphate resistant recombinant enzyme of the present invention. Whyard et al. report that the $K_m$ of the isolated enzyme towards malathion is about 11 μM (*See* Figure 3 of Whyard

(a) and Table V of Whyard (b)). In contrast, the $K_m$ of the presently claimed recombinant enzyme towards malathion is about 1.09 μM. (*See* compound E3W251L at Table 2 of Devonshire et al, Pesticide Biochemistry and Physiology (2003) 76:1-13, copy enclosed). (Compound E3W25IL is the recombinant enzyme of the present invention). Furthermore, the $K_{cat}$ of the molecule described by Whyard (b) is $45.7min^{-1}$ (*See*, for example, Table V of Whyard (b) ), whereas the $K_{cat}$ of the presently claimed enzyme is shown to be $220min^{-1}$ (See Table 2 of Devonshire et al.). Accordingly, the enzyme of the claimed invention has a $K_m$ for malathion which is approximately ten-fold higher than that of the prior art, and a K*cat* for malathion which is almost five-fold higher than that of the prior art. As a result, the recombinant enzyme of the claimed invention has almost a fifty-fold higher K*cat*/$K_m$ than that of the cited prior art (compare Table V of Whyard (b) with Table 2 of Devonshire *et al.*). Thus, it is clear that the isolated enzyme of the cited prior art is not the same as the recombinant enzyme of the claimed invention.

Moreover, as described by Whyard et al (a), the enzyme of the prior art was purified from both resistant and sensitive strains (See first two paragraphs of the abstract of Whyard (a)). As a result the authors concluded that the differences in malathion resistance and susceptibility between the two strains is due to a "quantitative rather than a qualitative change in the MCE of the two strains." (see Abstract of Whyard (a)). In contrast, the present specification discloses that the malathion hydrolyzing activity of the insects analyzed is due to a **qualitative** difference in the enzymes possessed by resistant strains when compared to susceptible strains. This is highlighted in Table 2 of the present specification where all susceptible strains comprised a wild-type amino acid at position 251, whereas in resistant strains this amino acid was mutated.

Finally, Whyard (a) reported that the enzyme of the prior art is found, *inter alia*, in the mitochondria of both resistant and susceptible flies. In contrast, Applicant has advised that the enzyme of the present invention does not comprise a mitochondrial targeting signal, and is aware of no evidence that suggests that the enzyme of the claimed invention is found in the mitochondria of *L. cuprina*. Since a mitochondrial targeting sequence is an inherent property of an polypeptide sequence, it is clear that the enzyme of the prior art is not the same as the enzyme of the claimed invention.

In conclusion, there are three distinct lines of evidence that the enzyme reported by Whyard is not the same as the enzyme of the claimed invention. The enzyme of the claimed invention has i) a significantly higher $K_m$ and $K_{cat}$ towards malathion when compared to the enzyme of the prior art; ii) the enzyme of the claimed invention confers resistance through a qualitative change in the amino acid sequence of the esterase, whereas the resistance mechanism of the prior art enzyme is over-expression of the molecule; and iii) the molecule of the claimed invention has a different sub-cellular localization than that of the prior art molecule, due to the absence of a mitochondrial targeting sequence in the claimed enzyme. The totality of the evidence clearly demonstrates that the recombinant enzyme of the claimed invention is not inherently the same as the molecule of the cited prior art.

Accordingly, the rejection of claims 9 and 13-18 under 35 U.S.C. § 102(b) over the cited prior art is respectfully traversed.

It is respectfully submitted that the present application, as amended above, is in condition for allowance, an early notification thereof being earnestly solicited.

To the extent necessary, a petition for an extension of time under 37 C.F.R. §1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

MCDERMOTT, WILL & EMERY

Judith L. Toffenetti
Reg. No. 39,048

600 13th St., N.W.
Washington, D.C. 20005-3096
(202) 756-8000
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